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# DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN FRESH WATER FISH

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## ABSTRACT

Validated sample preparation procedures and high resolution gas chromatography-high resolution mass spectrometry techniques were utilized for the quantitative measurement of 4 to 695 pg/g (ppt) levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2378-TCDD) in the edible portions of fish from the state of Michigan. The methodology and the analytical results are presented.

## INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2378-TCDD), one of 22 isomers of TCDD, has received a vast amount of publicity and has been the subject of a number of controversial issues during the past decade. Much of the concern has been generated as a consequence of its unique toxicological properties. For example, the acute oral $LD_{50}$ of 2378-TCDD is 0.6 to 0.7 µg/kg for the guinea pig (1). In addition, studies have shown it to be potentially carcinogenic (2), teratogenic (3), and fetotoxic (4) in several species of animals. However, the hazards to human health have not been well defined.

Low concentrations of 2378-TCDD, pg/g (ppt) to µg/g (ppm), have been formed as by-products in specific manufacturing processes utilizing 1,2,4,5-tetrachlorobenzene to produce 2,4,5-trichlorophenol, which in turn is the raw material for the synthesis of the herbicides 2,4,5-T and Silvex. TCDD isomers may also be formed if the tetrachlorobenzene is impure. The recent identification of TCDD isomers in a 2-chlorophenol product (5) indicates that TCDDs, including 2378-TCDD, may be formed in other unknown and unexpected chlorinated phenol processes due to impurities in the raw material or reaction characteristics. The use and disposal of millions of kilograms of synthetic chemical products and by-products contaminated with 2378-TCDD has provided a significant route for it to enter the environment. Another route for 2378-TCDD and TCDD isomers to enter the environment which involves combustion processes has also been reported (6,7,8,9,10) in recent years.

The analysis for ppt levels of 2378-TCDD is often complicated by the presence of relatively high concentrations (ng/g to µg/g levels) of naturally occurring compounds and chlorinated industrial pollutants. Therefore, efficient sample preparation procedures coupled with highly sensitive and specific mass spectrometry detection techniques are required for the isolation and quantitative measurement of ppt levels of 2378-TCDD. Specificity for 2378-TCDD requires the use of polar and non-polar high resolution WCOT columns coupled to mass spectrometry or sophisticated sample preparation procedures. Many analytical methods (11,12,13,14,15,16,17) have been reported. The comprehensive EPA analytical methodology for determination of ppt levels of 2378-TCDD described elsewhere (18) relies on high resolution gas chromatography (HRGC)-high resolution mass spectrometry (HRMS) to provide the required specificity for 2378-TCDD measurements.

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## EXPERIMENTAL

### Sample Collection

The fish were collected in Michigan in 1978 from the Tittabawassee, Grand and Saginaw Rivers, Lake Michigan, and from the Saginaw Bay under the direction of the U.S. Environmental Protection Agency. The species were channel catfish, carp, yellow perch, small mouth bass, lake trout, and sucker. The fish samples (whole fish and fillets) were shipped in a frozen state to the EPA Toxicant Analysis Center in Bay St. Louis, Mississippi, for sample preparation. A chain of custody protocol was maintained from the time of collection of the fish throughout completion of the analyses.

### Sample Preparation

The preparation involved digestion of sample with boiling alcoholic KOH followed by extraction with hexane, concentrated $H_2SO_4$ wash, alumina column chromatography cleanup, and concentration of the alumina column eluate to 60 µL prior to shipment of extracts for analysis.

### Quality Assurance (QA) Program

A QA program was initiated in advance of sample preparation and consisted of fortification of edible portions (5 to 10 g) of actual samples and QA samples with 2.5 to 10 ng of fully labelled $^{37}Cl_4$ 2378-TCDD for determination of analytical methodology efficiency. QA samples were also fortified with 0 to 1250 pg 2378-TCDD. The 60 µL extracts of actual samples and QA samples were submitted to the HRGC-HRMS laboratory in a blind fashion; i.e., there was no way for the mass spectroscopist to distinguish between actual samples and QA samples. The types of samples utilized for QA purposes were North Atlantic ocean perch, fish from other locations, laboratory grown fish, a previously analyzed positive fish from the Tittabawassee River, beef liver, and method "reagent" blanks.

### HRGC-HRMS Method of Analysis

A Varian 311A MS equipped with an eight channel hardware multiple ion selection (MIS) device and directly coupled to WCOT columns was used for these analyses. The sample extracts were subjected to HRGC-HRMS MIS analysis (18) for determination of analytical methodology efficiency and the quantitative measurement of 2378-TCDD. The exact masses and corresponding elemental compositions utilized in these measurements were: the m/z 318.9793, perfluorokerosene (PFK) reference mass; the 319.8965 $C_{12}H_4O_2{}^{35}Cl_4$ and 321.8936 $C_{12}H_4O_2{}^{35}Cl_3{}^{37}Cl$ corresponding to 2378-TCDD; and the 327.8847 $C_{12}H_4O_2{}^{37}Cl_4$, fully labelled 2378-TCDD internal standard. Peak height measurements obtained from the analysis of samples and the analysis of samples fortified with known amounts of $^{37}Cl_4$-TCDD and 2378-TCDD (standard addition technique) were used to determine the analytical methodology efficiency (percent recovery of $^{37}Cl_4$-TCDD internal standard), ppt levels of 2378-TCDD in the sample and the minimum limit of detection for 2378-TCDD. Typical amounts of quantification standards used in these analyses were 100 to 400 pg $^{37}Cl_4$-TCDD and 5 or 10 pg 2378-TCDD. The minimum limit of detection was defined as the amount of 2378-TCDD that would provide clearly defined peak shapes for the masses m/z 319.8965 and m/z 321.8936 in the correct isotopic ratio (0.79:1 ± 10%) and with a signal-to-noise ratio greater than 2.5 to 1. The sample weight, aliquot of total extract used in the analysis, analytical methodology efficiency, sample matrix effects and the electronic noise present in the time frame of measurement determined the minimum limit of detection for each sample.

### Analytical Criteria

The analytical criteria utilized for confirmation of TCDD are shown in Table 1. Each analysis had to satisfy the criteria I to V to be considered a confirmed positive sample. The supplemental criteria A and B and additional criteria were also applied to a representative number of fish samples; i.e.: simultaneous response of m/z 320, 322, and 324 to confirm the tetrachloro isotope ratio; simultaneous response of m/z 320, 322, 257, and 259 to confirm the $M^+$-COCl loss, which is indicative of TCDDs structure; HRGC-HRMS peak-matching analysis in real time at 10,000 mass resolution with PFK as reference to confirm the presence of the exact masses 319.8965 and 321.8935 corresponding to 2378-TCDD at correct retention time; and confirmation of

2378-TCDD in extracts derived from a highly specific "neutral" (acetonitrile partitioning) extraction and cleanup procedure.

TABLE 1

CRITERIA UTILIZED FOR CONFIRMATION OF 2378-TCDD RESIDUES

I. Capillary column HRGC-HRMS retention time of 2378-TCDD.

II. Chlorine isotope ratio of the molecular ion (m/z 320 and m/z 322).

III. Correct HRGC-HRMS multiple ion monitoring response for 2378-TCDD masses and $^{37}Cl_4$-TCDD mass (simultaneous response for elemental composition of m/z 320, m/z 322, and m/z 328).

IV. Correct response for co-injection of sample fortified with $^{37}Cl_4$-TCDD and 2378-TCDD standard.

V. Response of m/z 320 and m/z 322 must be greater than 2.5 times the noise level.

Supplemental criteria which were applied to a representative number of fish samples:
(A) COCl loss indicative of TCDD structure.

(B) HRGC-HRMS peak-matching analysis of m/z 320 and m/z 322 in real time to confirm the exact masses corresponding to TCDD elemental compositions.

## RESULTS AND DISCUSSION

A summary of analytical results for the quantitative measurement of 2378-TCDD in fish is shown in Table 2. An evaluation of the analytical results showed that 26 of 36 samples contained detectable quantities of 2378-TCDD. Concentrations ranged from 4 ppt to 695 ppt. Ten samples contained concentrations greater than 40 ppt. The highest concentrations were detected in catfish and carp (bottom feeders) and the lowest concentrations were detected in bass, perch, and sucker. The highest concentration was detected in a catfish collected from the Tittabawassee River, and the lowest concentration was detected in a composite of two suckers from commercial fishing waters in Saginaw Bay. Three additional compounds which satisfied the analytical criteria for TCDD isomers were detected in several fish samples at very low concentrations. One isomer exhibited the HRGC retention time identical with that of 1368-TCDD which was determined by co-injection of sample and the specific standard. The other two resolved isomers were not identified. 2378-TCDD was also detected in several fish from the Grand and Saginaw Rivers and Saginaw Bay.

Two other laboratories have also reported the presence of 2378-TCDD in fish collected from the Tittabawassee River. The results described in this paper represent the first reported analyses of fish from the Saginaw and Grand Rivers for 2378-TCDD residues. Confirmation of these results by another or several other laboratories would provide validity for these findings. The analytical results reported by Dow Chemical Company (8) for several species of fish from specific locations on the Tittabawassee River were in reasonable agreement with the results reported by this laboratory with but a few exceptions, i.e., the 695 ppt reported in Table 2. Kuehl et al. (19) detected the unique presence of tetra- through octa-chlorinated dibenzo-p-dioxins in fish collected from this river during a survey of several rivers and lakes. The method was not specific for 2378-TCDD but the estimated concentrations of TCDD were in the range of 0.5 ppb. Buser (20) also reported the presence of 2378-TCDD in a fish from the Tittabawassee River.

The source or sources, i.e., combustion process, manufacturing process or synthetic chemical products, of 2378-TCDD contamination in the Tittabawassee River fish is not known. Other laboratories, including this laboratory, have detected a distribution of TCDD isomers in effluents from specific types of combustion processes and the 2378-TCDD is usually a minor component. Laboratory studies necessary to determine any preferential retention of specific TCDD isomers by fish and other animals have not been reported. However, a study reported in 1979 (21) indicates that other chlorinated dibenzo-p-dioxin congeners with chlorine in the 2378 positions are preferentially retained by animal tissue.

TABLE 2

ANALYTICAL RESULTS FOR 2378-TCDD RESIDUES IN FISH

| Species | No. of Samples | No. of Positive Samples | Sample Preparation Efficiency[f] | TCDD Detected[g] (ppt) Low | High | Mean | TCDD Minimum Limit of Detection[g] (ppt) |
|---|---|---|---|---|---|---|---|
| Channel Catfish | 8 | 8[a] | 87 | 28 | 695 | 157 | 13 |
| Carp | 14 | 10[b] | 85 | 20 | 153 | 55 | 7 |
| Yellow Perch | 6 | 3[c] | 78 | 10 | 20 | 14 | 6 |
| Small Mouth Bass | 2 | 2[d] | 100 | 7 | 8 | 8 | 6 |
| Sucker | 4 | 3[e] | 92 | 4 | 21 | 11 | 4 |
| Lake Trout | 2 | 0 | 100 | 0 | 0 | 0 | 5 |

[a]Tittabawassee - 4, Saginaw - 2, Saginaw mouth - 1, Grand - 1.
[b]Tittabawassee - 5, Saginaw - 2, Saginaw mouth - 1, Grand - 2.
[c]Tittabawassee - 2, Saginaw mouth - 1 (composite of 2 fish for each analysis).
[d]Grand - 2.
[e]Tittabawassee - 2, Saginaw Bay - 1.
[f]Mean % recovery for 2.5 to 10 ng $^{37}Cl_4$-TCDD added to 5 or 10 g samples prior to sample preparation.
[g]Corrected for losses in sample preparation efficiency. ppt = parts per trillion.

Seventeen previously described QA samples were analyzed in this study(18). The mean percent recovery of 2.5 to 10 ng $^{37}Cl_4$-TCDD was 86 percent, and the mean percent accuracy obtained for 0 to 125 ppt 2378-TCDD was ± 15 percent. The number of QA samples with zero fortification levels of 2378-TCDD was very important in these analyses because of the large number of positive fish extracts which exhibited unusual and high concentrations of other chlorinated contaminants. Accurate results were obtained for a positive Tittabawassee fish sample fortified with a known amount of 2378-TCDD. It is significant that no false positive results were detected in the QA program.

Unusual and/or high concentrations of other co-extractable chlorinated contaminants were detected in the Michigan fish during the analyses for 2378-TCDD. The analyses of an extract of Tittabawassee River carp and a North Atlantic ocean perch are shown in Figures 1 and 2. The chlorinated contamination evident in the carp (Figure 1) did not present serious problems in the quantitative measurement for 2378-TCDD when criteria I through IV, Table 1, were utilized. Upon application of supplemental criterion (B), the exact masses of TCDD were confirmed and the masses representing the contamination were observed and evaluated. The contaminant masses were estimated to be resolved from TCDD masses by 20 to 70 millimass units. Only a fraction of the total intensity of these masses is detected and recorded in the HRGC-HRMS analysis. A typical analysis of QA sample extracts is shown in Figure 2. With the exception of the Tittabawassee River fish used for QA purposes, chlorinated contamination was not detected in QA samples.

After completion of the 2378-TCDD analyses, twelve fish extracts were pooled and subjected to HRGC-LRMS and HRGC-HRMS analyses. Tentative identifications were assigned to a number of the compounds: tetra-, penta- and hexa-chlorinated diphenylethers; a trichlorohydroxybenzylphenyl ether; tetrachlorodibenzofurans; a trichloroterphenyl; polychlorinated biphenyls; trichlorodibenzo-p-dioxins; and pentachlorodibenzo-p-dioxins. These identifications were based on the high mass region of the mass spectra (m/z 150 to m/z 500) and/or HRGC-HRMS determinations of the elemental composition of specific masses of each compound.

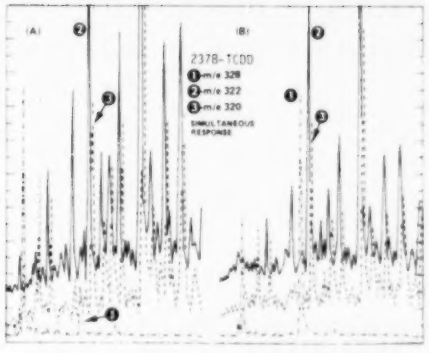


Figure 1. HRGC-HRMS multiple ion monitoring chromatogram obtained for (A) Tittabawassee River carp extract, 1 µl from 55 µl and (B) 0.5 µl of extract fortified with $^{37}Cl_4$-TCDD and 2378-TCDD. Experimental results: Retention time of $^{37}Cl_4$-TCDD and 2378-TCDD on 30 m x 0.25 mm i.d. SE-30 WCOT column was 17 min. 30 sec.; 80% recovery of $^{37}Cl_4$-TCDD; 93 ppt 2378-TCDD detected; 2378-TCDD detection limit, 6 ppt.

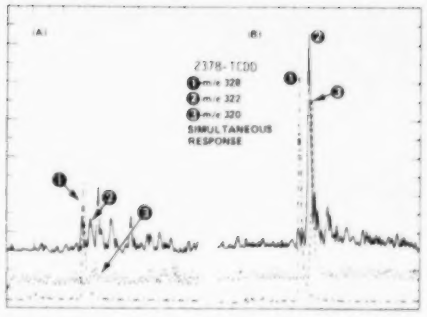


Figure 2. HRGC-HRMS multiple ion monitoring chromatogram obtained for (A) North Atlantic ocean perch extract, 2 µl from 55 µl and (B) 2 µl of extract fortified with $^{37}Cl_4$-TCDD and 2378-TCDD. Experimental results: Retention time of $^{37}Cl_4$-TCDD and 2378-TCDD on 30 m x 0.25 mm i.d. SE-30 WCOT column was 17 min. 30 sec.; 82% recovery of $^{37}Cl_4$-TCDD; 2378-TCDD not detected; 2378-TCDD detection limjt, 3 ppt.

In summary, the analytical results indicate that fish in a number of separate bodies of water in Michigan (Tittabawassee, Saginaw and Grand Rivers, and the Saginaw Bay) are contaminated at ppt levels with 2378-TCDD. The source or sources of the contamination is unknown. In addition to 2378-TCDD, other toxic compounds were qualitatively identified.

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